THE MALE AND FEMALE EGGS OF PHYLLOXERANS OF THE HICKORIES.

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The predetermination of sex in the egg has been demonstrated only where small male and large female eggs occur, and it is a very striking fact, so far overlooked I believe, that in these cases the difference in the male and female eggs is connected with the development of degenerate males. In *Dinophilus apatris* the male is smaller than the female, is degenerate, and the sexual organs appear to be precociously developed. In *Hydatina senta* the male is smaller than the female and degenerate. In the *Phylloxera* of the grape· and of the hickories the male is small, wingless, without digestive tract, and the sexual organs, as I shall show for one species at least, develop very early, so that the spermatozoa are fully formed before the male leaves the egg.

In *Dionphilus* it is believed that both the male and the female eggs are fertilized, but in *Hydatina* and in the phylloxerans the male and the female eggs are not fertilized. Sex in these cases is, therefore, determined independently of fertilization and preexists in the egg. In cases of this sort it would obviously be of great interest to discover what conditions determine that some eggs become males and others females. Is the difference, for example, connected with a visible difference of the nucleus, or of the cytoplasm? This question I believe I am able to answer, but the deeper-lying problem as to the causes that lead to the difference observable in the egg I have not fathomed.

The material for study was collected from the galls of the hickories in the spring and summer of last year, and included five or six species, of which three only will be mentioned here. The typical life history of these phylloxerans is the following: The fertilized egg, attached to the bark, hatches in the spring producing the stem-mother, who migrates to the young leaves and attaches herself to one spot on the under surface of the leaf, which becomes the center for the formation of a gall within which she becomes enclosed. She lays a large number of eggs inside

the gall that are all of one size, and give rise to the individuals of the second generation, which, developing wings in most species, ultimately leave the gall. These migrants contain two kinds of eggs, larger eggs that give rise to females, and smaller eggs that give rise to males. The eggs are deposited upon the bark

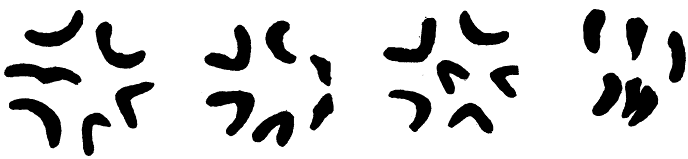

FIG. 1. *Phylloxera globosum.* Polar spindles of male and female eggs. The first figure (to the left) is from a large female egg; the second, from a small male egg; the third and fourth figures are from eggs whose size was not determined.

of the hickory. From them the male and female individuals are hatched. These soon pair and the female lays her single, large, winter egg on the bark of the tree. This egg hatches in the following spring, and produces the stem-mother.

A few species have a somewhat different cycle, and in one of them the male and the female eggs are laid within the gall itself. Owing to this condition a large number of the eggs in all stages of development can readily be collected. I have been fortunate enough to obtain a species of this sort, viz., *Phylloxera* sp. ? and have obtained an abundance of developing male and female eggs. The life history of a species of this sort according to Pergande is as follows: The stem-mother lays eggs that give rise to a generation of wingless forms corresponding to the winged migrants of other species. These wingless individuals contain large and small eggs which they deposit within the gall. From these eggs the minute males and females emerge. In a few cases a winged individual — a migrant — is found with the wingless individuals. It seems probable that the wingless condition is the secondary one, hence the occasional appearance of winged forms.

The Male and the Female Eggs of the Migrants. — The eggs are mature in the migrant before it leaves the gall, and the polar spindle is present. It is a difficult matter to find the spindle, and the chance is also small of getting one cut parallel to its equatorial plate. Nevertheless, I have found a fair num-

ber of such cases in which the number of the chromosomes could be counted with perfect accuracy. In *Phylloxera globosum* there are only six chromosomes in the polar spindle (Fig. 1), which makes it a very favorable object for study. In all eggs in which the chromosomes could be counted the same number was found,

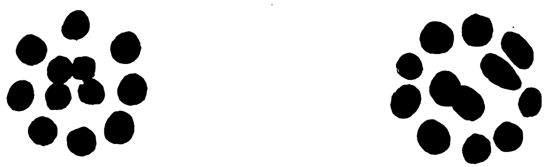

FIG. 2. *Phylloxera* sp. ? Left hand figure is a spermatogonial equatorial plate. Right hand figure is from a somatic cell of a male embryo.

and this is true both for male and for female eggs. In this respect there seems to be no difference between the two kinds of eggs.

In *Phylloxera* sp. ? the number of chromosomes in the male and female eggs seems to be twelve (Fig. 2). It is more difficult to count so many chromosomes with accuracy, at least in the eggs that I have so far seen ; but as the clearest cases observed showed twelve chromosomes, and as in other eggs eleven, or twelve, or thirteen seemed to be present, and since the num-

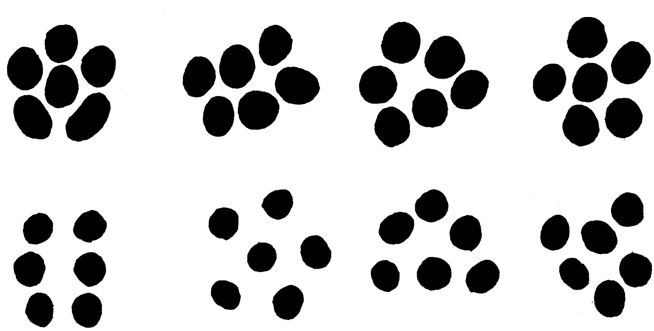

FIG. 3. *Phylloxera* sp. ? Spermatocyte divisions. Equatorial plates. Upper row, first spermatocyte divisions ; lower row, second divisions.

ber of chromosomes in the spermatocytes is definitely six (Fig. 3), there can be little doubt that the number in the polar spindle is twelve. In *Phylloxera caryæ-globuli* there are twenty-

two chromosomes present of very different sizes, as seen in the figures (Fig. 4).

In passing, it is worth while calling attention to the very different number of chromosomes found in these species of the same genus; species in fact, that are so similar that they can only be distinguished with great difficulty. It does not seem probable

FIG. 4. *Phylloxera caryae-globuli.* Polar spindle-equatorial plate of egg of migrants.

in the light of cases such as these that the absolute number of the chromosomes can be a matter of any special significance. If the chromosomes are all composed of the same identical substance it is difficult to account for their constancy in number and sizes in each species. If the chromosomes are different in composition, as the conditions just mentioned would seem to indicate, the differences can scarcely be associated with differences in the structures of the body, since closely similar individuals are produced in one case with six chromosomes and in the other with twenty-two chromosomes. This question is one of the most puzzling problems in the whole range of cytology at the present time, and it would be unwise to draw any conclusion from the meagre facts known to us. I wish only to indicate as a possible view that the chromosomes may be different chemically from each other, and yet at the same time this difference may have no connection with differences in the form of the body.

Further History of the Chromosomes. — Starting with the stem-mother the history of the chromosomes through the life cycle is as follows: The number of chromosomes present in the polar spindle of the eggs laid by the stem-mother is the somatic or whole number. The number in the somatic cells of the embryo that develops from this egg is also the whole number. The number in the polar spindle of the male and the female eggs is,

as has been stated above, the whole number ; and this is true for the somatic cells of both male and female embryos. In the spermatogonial cells the whole number of chromosomes is present, but in the two following spermatocyte divisions the half, or reduced number, occurs. I have seen the spindle in one winter egg, which seems to have only the half-number of chromosomes. It is evident, therefore, that the complete number of chromosomes is characteristic both for somatic and germinal cells throughout the life cycle, except for the winter egg and the spermatocytes, where the reduced number occurs preparatory to fertilization. After fertilization the number of the chromosomes would be the same as the whole number. Thus it is evident that there is no reduction in the number of the chromosomes in the parthenogenetic egg, but there is in the winter egg. The former is not, the latter is, fertilized. The results for *Phylloxera* are in these regards parallel to those that Stevens has recently obtained in the aphids.

The Cytoplasm of the Male and Female Eggs. — Since no discernible difference was detected between the chromosomes of the small male and the larger female eggs, I examined with some care the cytoplasm of these two kinds of eggs. In the male eggs there is less yolk and the center of the eggs is occupied by a clear mass of cytoplasm, while in the female egg there is more yolk and no central cytoplasm. I have not observed any other differences, and even those just noted can not be made out with certainty for all eggs, but represent the extremes of the series. Double and triple stains gave no differences that I could detect. In the ovaries the eggs showed no differences. In *Dinophilus*, according to Richard Hertwig, the large female eggs are formed by the union of several cells. It would be difficult to detect such a difference if it existed in the case of *Phylloxera*, since the ovarian eggs appear to be fused together at their inner ends, and each egg as it leaves the ovary remains attached by a cord of protoplasm to the fused center of the egg-mass.

There are striking differences in the development of the male and the female embryos, and these I have followed in some detail, but can not present the results at this time. One important difference must however be alluded to, namely, the precocious

development of the relatively enormous reproductive organs of the male. The testis is present as a large mass of cells at the time when the blastoderm is first laid down, and the spermatogonial divisions occur at this time. The two spermatocyte divisions occur when the embryonic plate is forming, and before the fibers appear in the nervous system. The size of the mass of spermatocyte cells is very large compared to the rest of the embryo, and it fills the greater part of one half of the egg. The spermatozoa are fully formed at the time when the embryo is developed.

The precocious development of the large testicular mass in the male egg suggests that a preëxisting mass of cytoplasm from which the testis develops may be present in the egg. The central mass of cytoplasm might supply such material, but I have not been able to make out any connection between the two. The result is due rather to the more rapid development of certain embryonic cells than to the presence of any large cytoplasmic mass of preformed material. The question arises whether we are to regard the large eggs that produce the females as doing so simply because they are large, or are they large because they are already female eggs? The latter alternative seems the more probable. In the light of certain recent experimental results, more especially those of Driesch and of Godlewski it seems highly probable that the *early* development of the embryo is due almost entirely to cytoplasmic influences. If this is true also in the case of the eggs of *Phylloxera*, then I think we may safely ascribe the difference in the size of the male and female eggs to the difference in the kinds of cytoplasm that are present when the egg is fully formed, so that the immediate determination of the sex is a cytoplasmic phenomenon. Whether this cytoplasmic difference can be traced to a preëxisting cytoplasmic basis, or to nuclear influence, or to the influence of external conditions is quite unknown, but in the absence of any nuclear differences it seem questionable whether we should assume that such exists.